Pestic. Sci. 1998, **52**, 268–274

Use of a Field-Scale Biofilter for the Degradation of the Organophosphate Insecticide Coumaphos in Cattle Dip Wastes

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(Received 14 September 1996; revised version received 7 July 1997; accepted 28 October 1997)

Abstract: Insecticide wastes generated from livestock dipping operations are well suited for biodegradation processes since these wastes are concentrated, contained, and have no other significant toxic components. A field-scale biofilter capable of treating 15 000-litre batches of dip waste containing the acaricide coumaphos was used to reduce the coumaphos concentration in two successive 11 000-litre batch trials from 2000 mg litre^{-1} to 10 mg litre^{-1} in approximately 14 days at 25–29°C. Removal of coumaphos from the biofilter effluent is a function of both physical filtration and biodegradation by the biofilter. However, stoichiometric increases in chloride levels in the effluent as coumaphos concentrations decreased confirmed that coumaphos was being degraded by the biofilter rather than just being filtered out. In subsequent 5500-litre batch experiments, the addition of a vitamin supplement to the biofilter-treated dip resulted in a further decrease in coumaphos concentration to approximately 1 mg litre^{-1}. Results from incubations of two representative Texas soils with biofilter-treated dip spiked with [*benzo*-U-^{14}C] coumaphos revealed that 32–36% of the spiked [^{14}C] coumaphos was mineralized in the soils after 110 days at 30°C. © 1998 SCI.

Pestic. Sci. **52**, 268–274 (1998)

Key words: pesticide; coumaphos; bioremediation; wastewater; ticks; biofilter

1 INTRODUCTION

The Veterinary Services Branch of the US Dept of Agriculture's Animal and Plant Health Inspection Service (APHIS) runs a Tick Eradication Program designed to prevent the re-introduction of cattle fever into the United States through ticks on cattle imported from Mexico, or on cattle in areas of the US where there is likely to be exposure to ticks from Mexico. The primary tool used in the eradication program is a series of dipping vats placed at border crossing points. Currently, the pesticide of choice for this application is the organophosphate coumaphos [*O*-(3-chloro-4-methyl-2-oxo-2*H*-1-benzopyran-7-yl)*O*,*O*-diethyl phosphorothioate; 'Co-Ral']. The operation on the US side of the border currently employs 42 vats each containing about 15 000 litres of coumaphos at a level of about 1600 mg litre^{-1}. The current disposal method for dip wastes is to pump the material into evaporation pits or waste lagoons located adjacent to the dip vats. Since many of the evaporation pits are unlined, the underlying soils are contaminated with high concentrations of coumaphos and its metabolites.

Coumaphos-containing dip waste is an excellent candidate for disposal by biodegradation since these wastes are concentrated, contained, and have no other significant toxic components. Shelton, Karns *et al.*[1–4] have shown that microbial consortia present in selected vats can be induced to mineralize coumaphos (Fig. 1). A field

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 Pestic. Sci. 0031-613X/98/$17.50. Printed in Great Britain

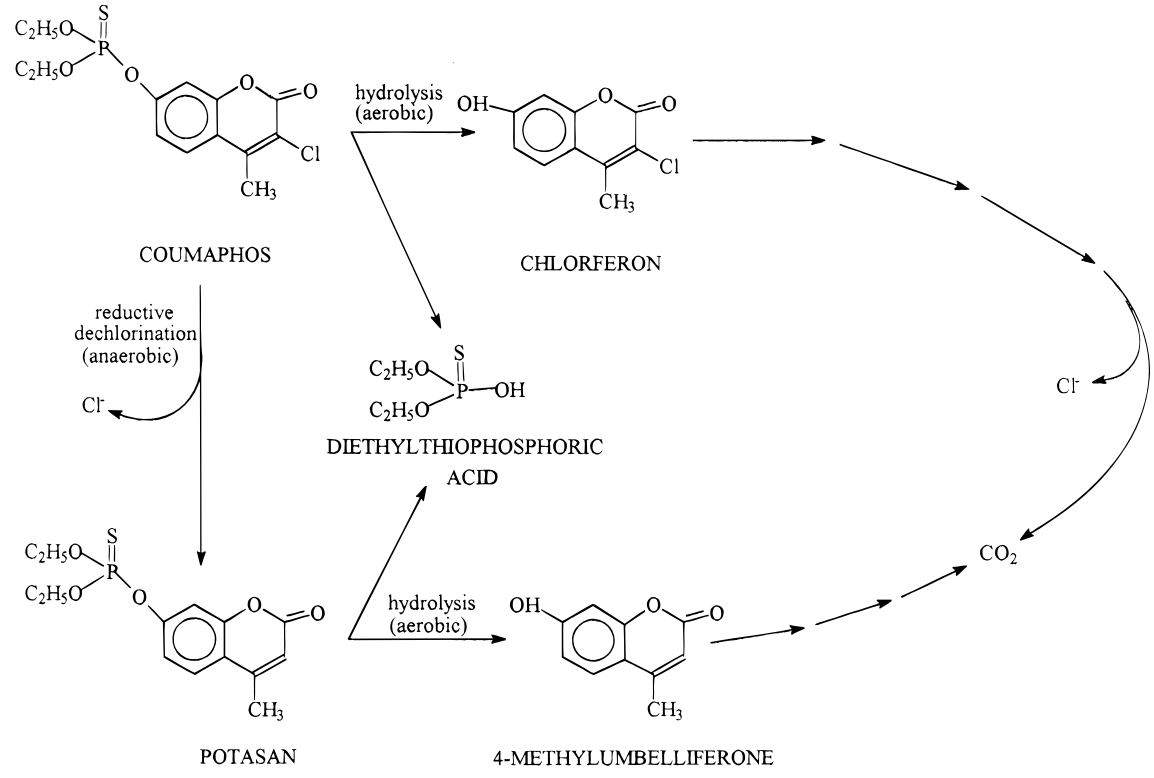

Fig. 1. Partial pathway of coumaphos metabolism under aerobic and anaerobic conditions.[1] Potasan is produced as a result of the reductive dechlorination of coumaphos under anaerobic conditions. Diethylthiophosphoric acid (DETP) and chlorferon are coumaphos hydrolysis products produced under aerobic conditions by parathion-hydrolase-producing bacteria. The products of this reaction are no longer acetylcholinesterase inhibitors.

trial was recently conducted using this approach to treat 15 000 litres of waste dip containing 1800 mg litre^{-1} coumaphos directly in the vat.[5] However, low ground temperatures and poor mixing conditions in the vat resulted in a slow biodegradation rate. Recently, we demonstrated that laboratory-scale gravel biofilters are effective in reducing coumaphos levels in treated dips to below 1 mg litre^{-1}.[6] Based on these results, a field-scale biofilter unit was constructed using a modified agricultural tank and polyethylene foam pad as the biofilter support. The objectives of this study were to: (1) test the efficacy of the field scale unit with 10 000 to 15 000-litre batches of dip waste; (2) test the effect of adding vitamin supplements to the biofilter; and (3) measure the mineralization rate of residual coumaphos present in biofilter-treated dip waste in two representative Texas soils.

2 EXPERIMENTAL METHODS

2.1 Chemicals

Analytical grade (99·6%) and formulated (42%) coumaphos, [*benzo*-U-^{14}C]coumaphos (sp. act. 21·1 mCi mmol^{-1}), potasan (94%), and chlorferon (97%) were gifts from the Animal Health Division, Bayer Corporation, Merriam, KS 66201. The [^{14}C]coumaphos was purified (99·5%) by thin-layer chromatography.[7] Sodium hydroxide was obtained from Fisher Scientific. For vitamin supplement experiments, generic daily multiple vitamin tablets were obtained at local grocery and drug stores. Each tablet contained 5000 units vitamin A (4750 IU as acetate, 250 IU as beta carotene), 60 mg vitamin C, 1·5 mg thiamine, 1·7 mg riboflavin, 20 mg niacin, 400 IU vitamin D, 30 IU vitamin E, 2 mg vitamin B_6, 0·4 mg folic acid, 6 μg vitamin B_{12} and 10 mg pantothenic acid. Prior to their addition to the biofilter reservoir, tablets were dissolved in water to give a final concentration of one tablet per 3·7 litres of biofilter liquid.

2.2 Analytical methods

For the measurement of coumaphos levels in biofilter-treated dips, samples (100 ml) were collected from the biofilter reservoir. Prior to sampling, the biofilter reservoir was vigorously aerated for ten minutes to resuspend coumaphos particles that had collected on the bottom and sides of the tank. Samples (100 ml) of treated dips were sent by overnight mail at ambient temperature to the Beltsville laboratory for coumaphos analysis by high performance liquid chromatography (HPLC).[6] Prior to HPLC analysis in the laboratory, small aliquots from these samples were either diluted five-fold with methanol (for samples containing levels of

coumaphos at or above 30 mg litre^{-1}) or concentrated after extraction with equal volumes of ethyl acetate (for samples containing levels of coumaphos below 30 mg litre^{-1}).[6] Chloride levels in the dips were determined using a Buchler chloridometer. Evolution of [^{14}C]carbon dioxide was quantified by liquid scintillation.[1]

2.3 Sampling sites and collection

Soil samples (approximately 1 kg each) were collected from two sites near the ARS laboratory at Mission, TX. These sites were in pristine areas that had not been exposed to any agricultural chemicals. Material was collected from the soil surface down to approximately 15 cm. After shipment of the samples back to the laboratory, each soil was homogenized by hand and stored in airtight plastic bags at 4°C. Soil analysis was performed by the Cooperative Extension Service of the University of Maryland at College Park. Table 1 provides each soil's characteristics. For field-scale biofilter experiments, batches of coumaphos dip waste (11 000–15 000 litres) were transported from APHIS dipping vats in Texas to the ARS field site in Mission, TX.

2.4 Biofilter components and operation

A 5·2 m × 3·1 m diameter fiber-glass agricultural storage tank (Red Ewald, Inc., Karnes City, TX) was modified for biofilter use by the addition of an open grating to support the biofilter (Fig. 2). The biofilter consisted of 14 layers of a 3·2-cm thick polyethylene foam pad (Water Garden, Chattanooga, TN). A Vertiflow series 1320 pump (Odessa, Inc., Houston, TX) was used to recirculate the dip from the reservoir to the biofilter pad at approximately 229 litre min^{-1}. The pH of the dip waste to be used on the biofilters was adjusted and maintained at 7·5–8·0 by the addition of sodium hydroxide pellets. In these field trials, approximately 13 kg of sodium hydroxide was needed initially to adjust the pH of each 11 000-litre batch of dip that had been previously amended with triple superphosphate fertilizer (61·4 kg 10 000 litre^{-1}).[5] In addition, approximately 12 kg of sodium hydroxide pellets was added to

TABLE 1
Characteristics of Samples of Two Texas Soil Types

				Soil composition (%)			
Soil sample	*Soil texture*	*pH*	*CEC*[a] *(meq)*	*Sand*	*Silt*	*Clay*	*Organic matter* (%)
Caliche	Sand	7·8	35·8	68	24	8	1·3
Pasture	Sandy loam	7·7	18·4	70	17	13	1·7

[a] Cation exchange capacity.

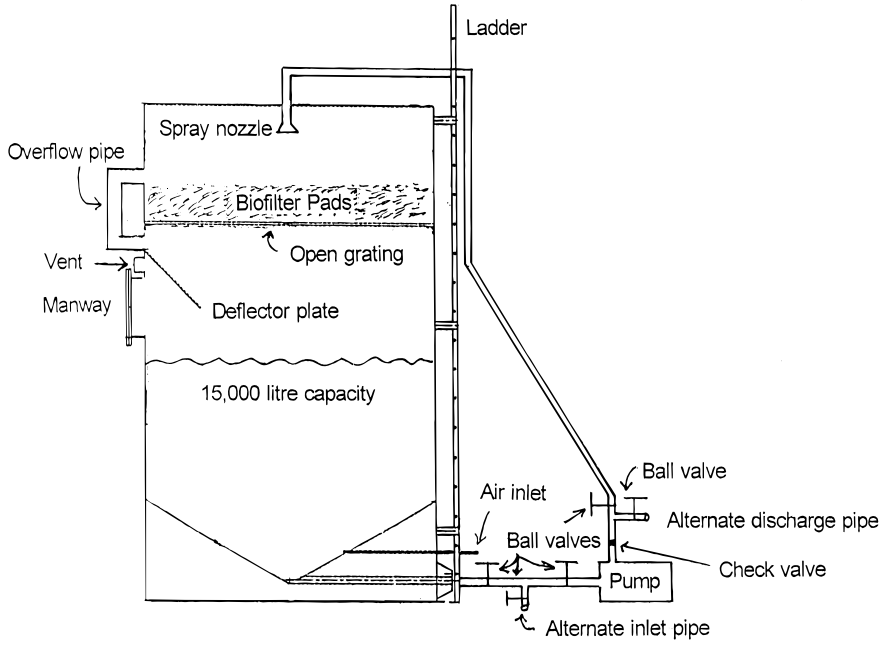

Fig. 2. Schematic drawing of biofilter components. A lightweight, mobile fiberglass tank (5·5 m × 3·2 m dia.) designed for agricultural uses was modified for biofilter use by the addition of an open grating that was used to support the biofilter. An open-weave polyethylene pad was used as the biofilter support.

each batch of biofilter-treated dip during the treatment period.

2.5 Soil incubations

Soil samples (40 g) were mixed with biofilter-treated dip (8 ml) containing approximately 10 mg litre^{-1} coumaphos and 0·001 mCi [^{14}C]coumaphos in 250-ml biometer flasks and incubated at 28–30°C on a rotary shaker at 120 rev min^{-1} for up to 110 days. The final moisture content of the soils was approximately 0·2 bar. To the flask to which no biofilter-treated dip was added, potassium phosphate buffer (50 mM, pH 7·0; 8 ml) was added in place of the dip. Flasks containing sterilized dip or sterilized soil were sterilized by autoclaving twice for 30 min at 125°C. One flask was used for each treatment. No attempt was made to trap potential volatile organic compounds.

3 RESULTS

3.1 Biofilter efficacy in treating coumaphos in cattle dip

Three field trials using the biofilter unit were conducted from September 1995 to February 1996 (Fig. 3). The first two trials used batches of waste dip containing the flowable liquid CoRal® formulation of coumaphos. The third trial used a batch of dip containing the wettable powder formulation of coumaphos. The temperatures of the dips ranged between 25 and 29°C during the first two trials, and between 20 and 25°C for the first 30 days of the third trial. During first two trials, coumaphos levels in the dips decreased two hundred-fold (2000 mg litre^{-1} to 8–10 mg litre^{-1}) within 15 days. No further significant decrease in coumaphos level was observed after longer treatment (Fig. 3). The biofilter became fouled after the third batch of dip was added and the coumaphos concentration leveled off at approximately 50 mg litre^{-1} (Fig. 3). The biofilter was then rinsed with

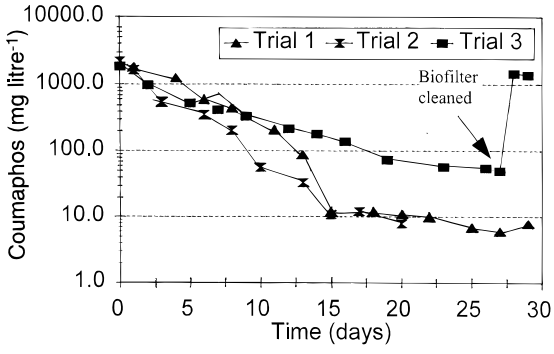

Fig. 3. Removal of coumaphos from three 11 000-litre batches of biofilter-treated dip. In the first two trials, coumaphos concentrations decreased from 2000 mg litre^{-1} to 10 mg litre^{-1} within 15 days at 25–29°C. Additional treatment time did not result in significant decreases in the concentration of coumaphos below 10 mg litre. The biofilter pad fouled during the third batch and was rinsed with biofilter effluent at the time indicated to release bound undegraded coumaphos.

treated dip from the reservoir to release the bound material back into the biofilter reservoir, and the system was restarted. Coumaphos levels after biofilter rinsing revealed that much of the coumaphos in the third batch had been trapped on the biofilter without being degraded before the biofilter fouled. After the biofilter unit was cleaned and restarted, coumaphos levels in the dip decreased from 1460 to 870 mg litre^{-1} before low ambient temperatures (5–15°C) essentially stopped further coumaphos degradation (not shown). During these and subsequent trials, we did not observe significant levels (<10 mg litre^{-1}) of known aromatic metabolites (potasan, chlorferon and methylumbelliferone) nor any new metabolites that absorb in the 210–320 nm range.

3.2 Use of chloride as a indicator of coumaphos biodegradation

The removal of coumaphos from the biofilter effluent is a function of both physical filtration and biodegradation by the biofilter. Previous results using laboratory-scale biofilters revealed that filtration of coumaphos was difficult to factor out when trying to measure the coumaphos biodegradation rate by the biofilter.[6] Fortunately, chloride is produced from the biodegradation of coumaphos and serves as an excellent soluble indicator of biofilter performance. Chloride levels increased stoichiometrically in the first two batches of biofilter effluent as coumaphos concentrations decreased (Fig. 4, panel A). Increases in chloride levels in the biofilter effluent beyond the expected stoichiometric amount were due to evaporative water loss during biofilter operation. During the first two trials, evaporative water losses were estimated to be approximately 20% after three weeks of treatment. During the third batch of cattle dip, chloride levels increased by only about two-thirds of the expected amount (3 mM of the 5 mM expected) before the biofilter fouled prior to cleaning (Fig. 4, panel B). Biofilter cleaning released approximately 1·5–2 mM more coumaphos than was expected from the chloride release results. Although evaporative water loss probably accounted for some of the discrepancy between chloride and coumaphos levels, these results suggest that some of the coumaphos from the previous two trials was trapped on the pads without being degraded. We estimated from laboratory studies,[6] that 6–10% of the first two batches of coumaphos loaded onto the biofilter was trapped on the pads without being degraded.

3.3 Effect of vitamin addition to the biofilter

To test whether it was possible to reduce biologically the final coumaphos concentration in biofilter-treated dips to below the levels achieved in the first two field

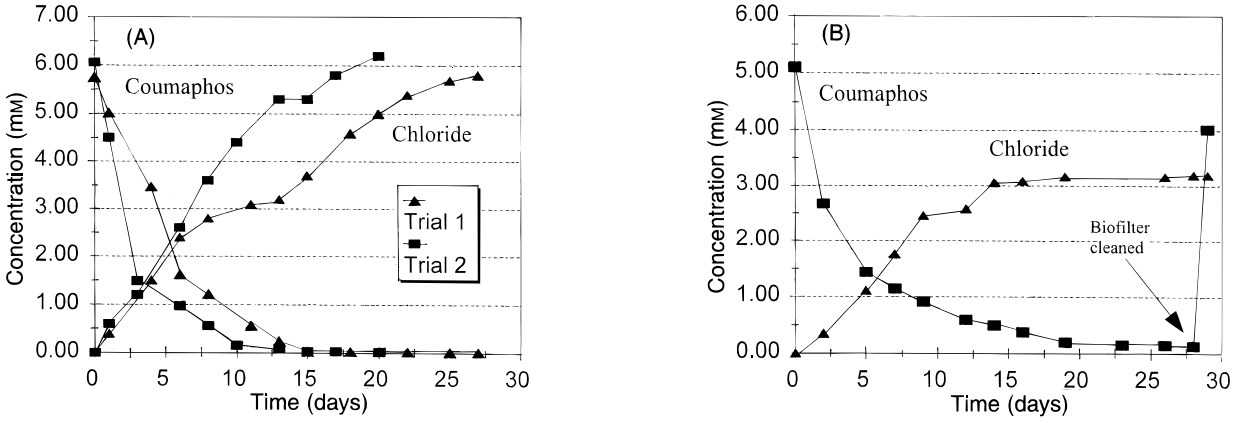

Fig. 4. Release of chloride from coumaphos during biofilter treatment. During the first two trials, chloride concentrations increased stoichiometrically as the coumaphos concentrations decreased (panel A). During the third trial, chloride release was less than stoichiometric because of biofilter fouling (panel B).

trials, two field trials that tested the effect of adding vitamins to the biofilter were conducted using identical 5500-litre batches of spent dip from one vat. As in the first two field trials, in trial 4 the coumaphos level in the biofilter-treated dip decreased to 5–7 mg litre^{-1} prior to adding the vitamin supplement (Fig. 5, trial 4). However, within five days after adding the supplement, the coumaphos level decreased further to 1·2 mg litre^{-1}. It should be noted that, in this trial, the low rates of coumaphos degradation were probably due to very low (5–15°C) ambient temperatures (Ref. 5 and Mulbry, unpublished results). In trial 5 (conducted at ambient temperatures of 25–29°C), the coumaphos level decreased to 1 mg litre^{-1} before vitamins were added, suggesting that the vitamin-stimulated growth and/or activity persisted on the biofilter between batches of dip (Fig. 5, trial 5).

3.4 Mineralization in soil of residual coumaphos in biofilter-treated dip

Previous studies have shown that coumaphos is persistent in soil, with a half-life of 300 days.[1] However, the presence of coumaphos-degrading micro-organisms and nutrients present in biofilter-treated dip could affect the soil biodegradation rate of residual coumaphos present in these treated dips. Soil incubation experiments using two representative Texas soils were conducted to measure the biodegradation rate of residual coumaphos present in biofilter-treated dip (Fig. 6). In the first experiment, biofilter-treated dip from the first field trial (containing 8 mg litre^{-1} coumaphos and spiked with [^{14}C]coumaphos) was added to biometer flasks containing each soil, and the evolution of [^{14}C]carbon dioxide was measured after incubation at 28–30°C (Fig. 6, panel A). The results from that experiment were similar for both soils, with [^{14}C]carbon dioxide accounting for 32–36% of the added radioactivity after 110 days. Previous experiments in which [^{14}C]coumaphos was completely mineralized in soil slurries containing coumaphos-degrading consortia showed that 60% of the added radioactivity was evolved as [^{14}C]carbon dioxide.[1] By comparing these results we estimate that approximately half of the residual coumaphos in biofilter-treated dip was mineralized in the present experiments after 110 days. A second experiment was conducted to estimate the relative contributions of the micro-organisms and nutrients in the biofilter-treated dip to the coumaphos biodegradation rate in soil (Fig. 6, panel B). In this experiment, sterile and non-sterile soil samples were amended with sterile and non-sterile samples of biofilter-treated dip. In addition, a sample of non-sterile soil was incubated with [^{14}C]coumaphos that had been mixed with phosphate buffer rather than biofilter-treated dip. As expected, this experiment showed that abiotic generation of carbon dioxide from coumaphos was negligible. Less than 2% of added radioactivity was evolved as [^{14}C]carbon dioxide from an incubation containing sterile dip and sterile soil. Indigenous micro-organisms present in non-sterile soil samples were capable of generating a small amount (6% of the added radioactivity) of [^{14}C]carbon dioxide from coumaphos that had been diluted with phosphate buffer rather than biofilter-treated dip.

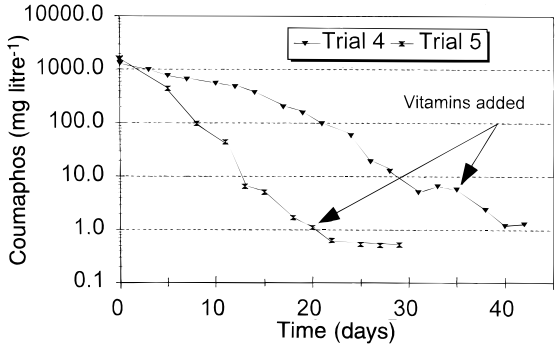

Fig. 5. Effect of vitamin addition to biofilter-treated dip during field trials 4 and 5. A multi-vitamin supplement was added to the biofilter reservoir at the times indicated in the field trials. In trial 4 the initial low rates of coumaphos degradation were probably due to low (5–15°C) ambient temperatures. In trial 5 the rate of coumaphos degradation was much faster (comparable to field trials 1 and 2) because of higher (25–29°C) ambient temperatures.

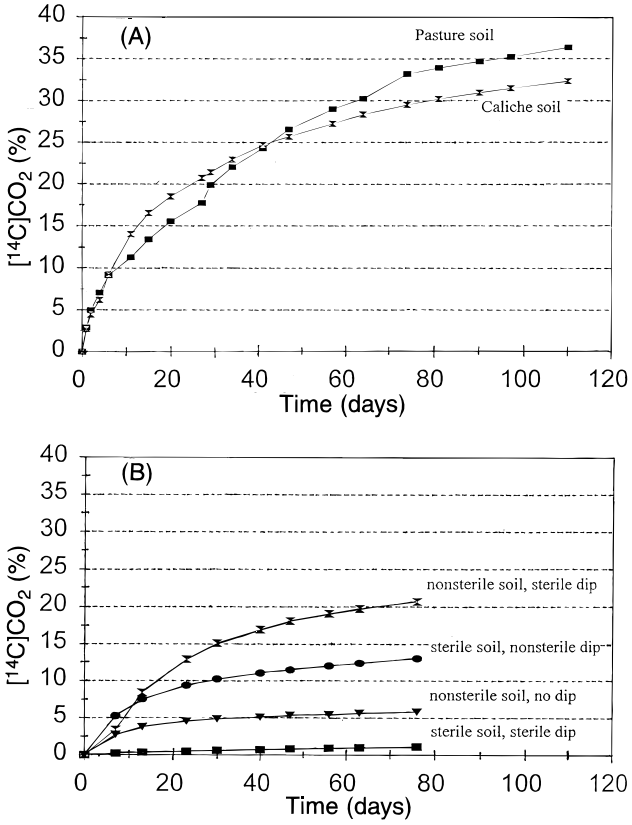

Fig. 6. Production of [^{14}C]carbon dioxide from [^{14}C]coumaphos using two representative Texas soils and biofilter-treated cattle dip. Panel A shows the results of a soil incubation experiment in which biometer flasks containing nonsterile Texas soils were amended with nonsterile biofilter-treated cattle dip and [^{14}C]coumaphos. Panel B shows the results of a soil incubation experiment in which biometer flasks containing pasture soil (sterilized or nonsterile) were amended with phosphate buffer or biofilter-treated cattle dip (sterilized or nonsterile) and [*benzo*-^{14}C]coumaphos.

However, the addition of either dip nutrients alone (present in sterilized dip) or dip micro-organisms and nutrients (in non-sterile dip) to soil samples significantly increased the rate and extent of coumaphos mineralization (21% and 13%, respectively) relative to the buffer and abiotic control incubations (Fig. 6, panel B). These results suggest that micro-organisms present in the soil, those present in the dip, and the nutrients in the treated dip all contribute to the increased coumaphos biodegradation rate in these soils.

4 DISCUSSION

Laboratory-scale biofilters using gravel as the biofilter matrix were able to reduce the coumaphos levels in treated dips to less than 0·1 mg litre^{-1} in 7–10 days at 28°C.[1] Comparable biofilter units using the polyethylene pads were much less efficient, yielding coumaphos levels of 10 mg litre^{-1} and 0·6 mg litre^{-1} after 16 and 25 days, respectively (W. Mulbry, unpublished results). However, these lightweight pads are easily cleaned and their low density allows for the use of inexpensive fiberglass or plastic tanks for scaled-up units. The performance of the field-scale biofilter approximated that of laboratory-scale units with regard to treatment time, but did not achieve coumaphos levels lower than 8–10 mg litre^{-1} even after 29 days.

Measurement of the coumaphos biodegradation rate in this system is complicated by the filtration of particulate coumaphos by the biofilter. However, laboratory studies using a variety of dips from different sites in shake-flask cultures revealed a stoichiometric increase in chloride concentration as coumaphos concentrations decreased (W. Mulbry, unpublished results). In addition, the spiking of these cultures with additional coumaphos led to corresponding increases in chloride concentrations (W. Mulbry, unpublished results). The use of chloride as an indicator of coumaphos degradation in these field trials resulted in slight overestimates of the coumaphos degraded because of evaporative water losses during the trials. However, the near-stoichiometric chloride increases in trials 1 & 2 suggest that no significant amount of coumaphos was being lost because of binding to the biofilter, tank walls, or piping. The trial 3 results are complicated by the trial being essentially shut down by low winter temperatures, but are important because they confirm the chloride data in demonstrating that the biofilter system degraded the coumaphos from trials 1 & 2 rather than just filtering it out.

To test whether it was possible to reduce biologically the final coumaphos concentration in biofilter-treated dips to below the levels achieved in the first two field trials, laboratory experiments were performed in which a variety of nutritional supplements, oils and detergents were added to shake flasks containing biofilter-treated dips from field trials 1 and 2 (containing 8–10 mg litre^{-1} coumaphos). These experiments revealed that one or more vitamins contained in generic daily vitamin tablets stimulated the growth and/or enzymatic activities of the microbial consortia responsible such that final coumaphos levels in coumaphos-degrading cultures dropped from 5–10 mg litre^{-1} in unsupplemented cultures to 1 mg litre^{-1} in vitamin-supplemented cultures (data not shown). Thus far, identification of the responsible vitamin(s) has been inconclusive. The concentration of multi-vitamin stock needed to stimulate this microbial growth and/or enzymatic activity varied between different batches of dip waste; however, a final concentration of one tablet per 3·7 litres consistently showed the desired effect in all batches. Experiments testing the effect of adding vitamin supplements to the field-scale biofilter showed that final treatment levels of 1 mg litre^{-1} are readily achievable. Other less expensive vitamin sources will be tested in future experiments.

Although we have not established the basis of the vitamin effect, Shelton & Somich[2] previously isolated a

stable two-member bacterial consortium from spent dip that was capable of metabolizing coumaphos. One of these strains (strain B-4) was able to metabolize one or more chlorinated intermediates of coumaphos and had an unspecified vitamin requirement for growth. It will be interesting to test whether strain B-4 is present in our biofilters and to investigate its role in decreasing coumaphos levels in the range of less than 10 mg litre^{-1}.

Disposal of the final biofilter effluent containing 1–10 mg litre^{-1} coumaphos is an important issue, since previous studies have shown that coumaphos is very persistent in soil and repeated land disposal of biofilter-treated dip might lead to ever-increasing coumaphos levels in treated soils. However, our results with two representative Texas soils show that the residual coumaphos present in biofilter-treated dip is much less persistent than untreated coumaphos. Nutrients and micro-organisms present in the treated dip, as well as the indigenous soil micro-organisms all contribute to this increased biodegradation rate.

Based on these results, the USDA/APHIS program has adopted this technology for the treatment of coumaphos dip waste. The US EPA has proposed that biofilter-treated dip containing approximately 10 mg litre^{-1} coumaphos can be applied directly to non-agricultural land or contained in concrete-lined evaporation tanks. Studies are underway to minimize biofilter fouling, optimize the flow rate through the biofilter, and characterize the parameters that limit further decreases in coumaphos levels in the biofilter effluent.

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